Clinical and Genetic Characteristics of Type 2 Diabetes With and Without GAD Antibodies

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The aim of the study was 1) to establish the prevalence of GAD antibodies (GADab) in a population-based study of type 2 diabetes in western Finland, 2) to genetically and phenotypically characterize this subgroup, and 3) to provide a definition for latent autoimmune diabetes in adults (LADA). The prevalence of GADab was 9.3% among 1,122 type 2 diabetic patients, 3.6% among 558 impaired glucose tolerance (IGT) subjects, and 4.4% among 383 nondiabetic control subjects. Islet antigen 2 antibodies (IA2ab) or islet cell antibodies were detected in only 0.5% of the GADab+ patients. The GADab+ patients had lower fasting C-peptide concentrations (median [interquartile range]: 0.46 [0.45] vs. 0.62 [0.44] nmol/l, P = 0.0002) and lower insulin response to oral glucose compared with GADab- patients. With respect to features of the metabolic syndrome, the GADab+ patients had lower systolic (140 [29.1] vs. 149 [26.0] mmHg, P = 0.009) and diastolic (79.2 [17.6] vs. 81.0 [13.1] mmHg, P = 0.030) blood pressure values, as well as lower triglyceride concentrations (1.40 [1.18] vs. 1.75 [1.25] mmol/l, P = 0.003). GADab+ men had a lower waist-to-hip ratio compared with GADab- patients. Compared with GADab+ patients and control subjects, the GADab+ patients had an increased frequency HLA-DOB1*0201/0302 (13 vs. 4%; P = 0.002) and other genotypes containing the *0302 allele (22 vs. 12%; P = 0.010). However, the frequency of these high-risk genotypes was significantly lower in GADab+ type 2 patients than in type 1 diabetes of young or adult onset (0201/0302 or 0302/X: 36 vs. 66 vs. 64%, P < 0.001). The GADab+ type 2 group did not differ from control subjects with respect to genotypes containing the protective DQB1-allies *0602 or *0603, nor with respect to the type 1 high-risk genotype in the IDDM1 (Hph1 +/-). We conclude that GADab+ patients differ from both GADab- type 2 diabetic patients and type 1 diabetic patients with respect to β-cell function, features of the metabolic syndrome, and type 1 diabetes susceptibility genes. Further, we propose that LADA be defined as GADab positivity (>5 relative units) in patients older than 35 years at onset of type 2 diabetes. Diabetes 48:150-157, 1999

A subgroup of patients diagnosed with type 2 diabetes has circulating antibodies to islet cell cytoplasmic antigens (ICA) (1-4) and more frequently to GAD (GADab) (5-7). Several studies have shown that positivity for ICA (3,4,8) or GADab (5-7, 9,10) correlates with insulin deficiency or relative insulin requirement in Caucasians, and also in Asians (11,12). Fifty percent of newly diagnosed GADab+ patients developed relative insulin deficiency, defined as glucagon-stimulated C-peptide concentration <0.7 nmol/l, after 10 years compared with 3% of GADab- patients (10). In consequence, 52% of GADab+ patients required insulin therapy after 6 years in the U.K. Prospective Diabetes Study (UKPDS) (7). Autoantibody positivity together with the subsequent development of insulin deficiency lead to introduction of the eponym latent autoimmune diabetes in adults (LADA) for this subgroup (5), which now has been included in the proposal for the new World Health Organization (WHO) criteria for diabetes as its own subgroup (13).

However, except for the UKPDS (7), the studies on LADA have been small and, at least partly, restricted to hospital outpatient clinics, which may have led to selection of more serious cases. The data on features of the metabolic syndrome in LADA are limited to one small study (14), although the patients with LADA may be indistinguishable from type 2 diabetes at onset of disease. The key question is whether LADA represents a late manifestation of type 1 diabetes or whether it can be considered a unique disease entity. One way to approach the problem is—in addition to comparing the phenotype between patients with LADA, type 1 diabetes, and type 2 diabetes—study whether LADA patients share a genetic background with type 1 diabetic patients.

Of the familial risk for type 1 diabetes, 42% has been ascribed to the IDDM1-locus in the HLA Class II region on chromosome 6p21, and 10% has been ascribed to the IDDM2-locus in the promotor region of the insulin gene on chromosome 11p15.5 (15). In addition, several other gene loci have been suggested to contribute to the genetic susceptibility to type 1 diabetes (16-23). Whether the type 1 diabetes susceptibility genes play a role in the etiology of polygenic type
RESEARCH DESIGN AND METHODS

Subjects. The subjects participated in the Botnia study in Western Finland, into which patients with type 2 diabetes and their family members have been recruited since 1990 (27). Nondiabetic spouses without family history of diabetes served as control subjects. Glucose tolerance was assessed according to the revised WHO and American Diabetes Association criteria (28). Thus, subjects with fasting blood glucose (FBG) ≥6.1 mmol/l or 2-h blood glucose ≥10 mmol/l during an oral glucose tolerance test (OGTT) were diagnosed with diabetes, and subjects with FBG <5.5 mmol/l and 2-h blood glucose <6.7 mmol/l as normal glucose tolerance, and subjects with intermediate blood glucose levels as impaired glucose tolerance (IGT). After exclusion of families with maturity-onset diabetes of the young (29), GADabs were analyzed for 2,063 subjects, including 383 control subjects, 558 IGT subjects, and 1,122 patients diagnosed with type 2 diabetes.

The clinical characteristics were compared with those in 194 patients (108 men, 86 women) with classic type 1 diabetes, including 82 patients from the Botnia Study (the age at onset of diabetes was ≤20 years in 50 and ≥20 years in 32 patients) and 112 patients diagnosed before the age of 35 years who attended the Jakstads Hospital outpatient clinic located in the Botnia region (30). Their mean age was 36.5 [20.8] years; age at onset of diabetes was 15 [14.8] years; duration of diabetes 6.5 ± 6.4 years, BMI 29.3 ± 4.5 kg/m². Data are % unless otherwise indicated. * P < 0.0001; † P < 0.001; ‡ P < 0.01; § P < 0.05; ¶ P < 0.1; || P < 0.05 (high GADab vs. other groups).

Antibody tests. GADab and islet antigen 2 antibodies (IA2ab) were measured by a radioimmunoprecipitation method employing 35S-labeled recombinant human GAD65 produced by in vitro transcription/translation (31,32). The Escherichia coli clones with complementary DNA for full-length human GAD65 or intracellular domain of IA2 were gifts from Drs. Allan E. Karlsen and Catherine E. Grubin, University of Washington (Seattle, WA) and Dr. Michael Christlieb, Kings College (London, U.K.), respectively. The results are expressed as relative units (RU): RU = (sample cpm – mean cpm of 3 negative controls)/cpm of a positive internal reference serum – mean cpm of 3 negative controls) × 100. The cutoff limit for positivity was 5 RU for GADab and 2.5 RU for IA2ab, which represent mean + 3 SD of 296 (GADab) or 155 (IA2ab) healthy Finnish control subjects. At the Combined Autoantibody Workshop (33), the specificity and sensitivity of the GADab assay were 99 and 75%, respectively.

ICA (34) and parietal cell antibodies (PCAs) (8) were determined by an indirect immunofluorescence technique. Anti-thyroid microsomal antibodies (TMSAs) and thyroglobulin antibodies (TGAs) were measured using Serodia-ATM or -ATG particle agglutination kits, respectively (Fujirebio, Tokyo, Japan) (8). The cutoff value for ICA was 5 Juvenile Diabetes Foundation units. The screening titers for PCA, TMSA, and TGA were ≥1:10, ≥1:400, and ≥1:100, respectively. ICA, PCA, TMSA, and TGA tests were performed for the first 517 type 2 diabetes patients recruited in the study and for all GADab+ patients for whom serum was available (Table 1). Of the control subjects, 1,122 (0.8%) was positive for ICA (0/36 M, 186 W), and 11/15 (9.6%) were positive for PCA (2/29 M, 9/86 W), 12/105 (11.4%) for TMSA (2/27 M, 10/78 W), and 4/105 (3.8%) for TGA (0/27 M, 4/78 W).

Metabolic measurements. An OGTT was performed for all subjects ≥15 years old who had FBG <10 mmol/l and were not treated with insulin. After 12 h of overnight fasting, the subjects ingested 75 g of glucose in a volume of 300 ml. Samples for measurements of blood glucose and serum insulin were drawn at 0, 10, 30, 60, and 120 min. Blood glucose was measured with a hexokinase method with a coefficient of variation (CV) of <1% (Boehringer Mannheim, Mannheim, Germany). Serum insulin concentrations were measured by a double-antibody radioimunoassay (Pharmacia, Uppsala, Sweden) with an interassay CV of 5%.

FS C-peptide concentrations were measured in duplicate by a radioimmunoassay with an interassay CV of 9% (35). HbA1c concentrations were measured by high-pressure liquid chromatography. The reference values for the assay were 5–7%. Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured on a Cobas Mira analyser (Hoffman LaRoche, Basel).

HLA-DQB1-genotypes. The second exon of the DQB1 gene was amplified by polymerase chain reaction (PCR) followed by dot-blotting onto positively charged nylon membranes (Hybond-TM-N+; Amersham, U.K.) and hybridization with sequence-specific oligonucleotide probes at 54°C in 50 mmol/l Tris-base, 2 mmol/l EDTA, 0.1% SDS, 3 mol/l tetramethylammonium chloride, 5X Denhardt’s, and 0.1 mg/ml herring sperm DNA. The sequence-specific oligonucleotide probes originating from the 11th International Histocompatibility Workshop (36) were labeled with digoxigenin (DIG Oligonucleotide 3’-End Labelling Kit; Boehringer Mannheim), and the bound probes were detected with alkaline phosphatase conjugated antidigoxigenin antibody and chemiluminescent substrate CSPD (DIG Luminescent Detection Kit; Boehringer Mannheim). The three DQB1 probes were used to distinguish the DQB1 alleles *0201, *0302, and either *0201 or *0603 (*0602[3]). Genotypes are presented as 0201/0302, 0201/X, 0302/X, 0201/0603, 0203/0602(3), 0602(3)/X, or X/X, where X could mean either a homozygous allele or any allele other than 0201, 0302, or 0603[3].

Hph1-polymorphism in the insulin gene. We used a restriction fragment length polymorphism method involving digestion of the PCR-amplified DNA (37) with Hph1 according to manufacturer’s instructions (Amersham, Buckinghamshire, U.K.) to produce fragments of 229 bp + 125 bp (+/-genotype), or 190 bp + 125 bp + 39 bp (+/+genotype).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Frequency of ICA, IA2ab, PCA, TMSA, and TGA in relation to GADab levels in patients diagnosed with type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative GADab (&lt;5 RU)</td>
</tr>
<tr>
<td>n (M/W)</td>
<td>517 (221/296)</td>
</tr>
<tr>
<td>IA2ab</td>
<td>0.5</td>
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<tr>
<td>ICA</td>
<td>0.6</td>
</tr>
<tr>
<td>PCA</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>9</td>
</tr>
<tr>
<td>Women</td>
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<td>TMSA</td>
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<tr>
<td>TGA</td>
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<tr>
<td>Men</td>
<td>3.2</td>
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<tr>
<td>Women</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Data are % unless otherwise indicated. * P < 0.00001 (all three groups); † P < 0.0001 (high GADab vs. other groups); ‡ P = 0.056 (high GADab vs. other groups); ¶ P = 0.002 (high GADab vs. other groups).
Statistical analysis. The statistical significance of the differences between group frequencies was tested by the χ² test (with Yates correction when appropriate) or Fisher's exact test. The differences in continuous variables between groups were tested by the Mann-Whitney test, the Kruskall-Wallis test for group means, or GLM analysis of variance using either the BMDP or SOLO statistical software (Biomedical Data Processing, Los Angeles, CA). Logarithmic transformation of data was used for the covariate analysis. Incremental glucose and insulin areas under the curve during the OGTT were calculated by the trapezoidal rule. For the statistical analysis, the insulin and C-peptide concentrations were adjusted for age, BMI, and duration of diabetes. The blood pressure and lipid data were adjusted for age and BMI. Data were analyzed separately for men and women, but the sex-specific data are given only when differences between the sexes were seen. Data are given as means ± SD or medians [75–25% interquartile ranges]. The P values are given uncorrected in the text and both uncorrected and corrected for the number of alleles/genotypes analyzed in the tables.

RESULTS

Prevalence of autoantibodies. GADabs were found in 104 of 1,122 patients diagnosed with type 2 diabetes (9.3%), 20 of 558 subjects with IGT (3.6%), and 17 of 383 control subjects (4.4%) (P < 0.0001). The frequency of GADab was significantly higher in patients who had been ≤45 years old at onset of diabetes than in patients >45 years old (19.3 vs. 8.2% P = 0.0001; Fig. 1). After an age of 45 years at onset, the frequency of GADab was rather stable and uninfluenced by the age at onset. The frequency and levels of GADab were similar between men and women in both diabetic and nondiabetic subjects. The frequency or level of GADab was not associated with the duration of diabetes.

The 104 GADab+ type 2 diabetic patients came from 96 families. Thus, eight families included two GADab+ patients who were first-degree relatives. Seven GADab+ patients had a first-degree relative with type 1 diabetes, and three had a second-degree relative with type 1 diabetes.

IA2abs and ICA were detected in 17 and 16% respectively, of the GADab+ patients, whereas they were rare (0.4 and 0.5%) in type 2 diabetic patients without GADab. Table 1 shows the antibody frequencies according to the level of GADab. The GADab+ patients were divided into tertiles of GADab positivity, and the patients in the highest tertile (>38 RU) were compared with those belonging to the two lower tertiles (≥5–38 RU) or without GADab (≤5 RU). Both IA2ab and ICA were more common in patients with high GADab levels compared with the group with intermediate GADab levels (IA2ab: 33 vs. 10% P = 0.009; ICA 43 vs. 1.4% P < 0.00001).

Of the other organ-specific autoantibodies, PCAs were increased in frequency in men with high GADab levels and TMSAs were increased in women with high GADab levels, whereas the overall frequencies of these antibodies were not associated with GADab positivity. The frequency of thyroid disease was not associated with GADab positivity (3 vs. 9 vs. 8% P = 0.59).

Clinical comparison of GADab+ and GADab- individuals IGT and control subjects. Table 2 shows the clinical characteristics for the groups according to glucose tolerance. No difference was seen between either GADab+ and GADab- control subjects or GADab+ and GADab- IGT subjects with respect to age, BMI, FBG, incremental insulin area, or lipid concentrations.

Patients diagnosed with type 2 diabetes. The GADab+ patients were between 28 and 83 years at diagnosis of diabetes, with a median age at onset slightly lower than that for the GADab- patients (58.5 [19.0] vs. 63.0 [15.0] years; P = 0.002) (Table 2). In the GADab+ group, 4.8% were diagnosed before the age of 35 years and 13.5% before the age of 40 years, compared with 2.6 and 5.4% in the GADab- group. The fasting glucose and insulin levels did not significantly differ between the antibody-positive and antibody-negative groups, but the GADab+ patients had lower FS C-peptide concentrations (0.46 [0.45] vs. 0.62 [0.44] nmol/l, P = 0.0002) (Table 2). During the OGTT, no difference was seen in the glucose response, but the insulin response was lower in the GADab+ compared with the GADab- patients (P = 0.039). In accordance with the lower C-peptide and insulin concentrations, the GADab+ patients were assigned insulin therapy more often (30 vs. 12%, P < 0.0001) and earlier (median duration of diabetes: 5.0 [10.3] vs. 11.0 [8.8] years, P = 0.009) than were the GADab- patients.

The GADab+ patients had lower systolic (140 [29.1] vs. 148 [26.0] mmHg, P = 0.009) and diastolic (79.2 [17.6] vs. 81.0 [13.1] mmHg, P = 0.009) blood pressure values, as well as lower triglyceride concentrations (1.40 [1.18] vs. 1.75 [1.25] mmol/l, P = 0.003) (Table 2). The HDL cholesterol concentrations were higher in GADab+ than in GADab- patients (P = 0.019), but the difference was not statistically significant when corrected for BMI (1.26 [0.36] vs. 1.19 [0.38] mmol/l, P = 0.055). The total cholesterol concentrations were similar between the two groups. Also, GADab+ men had a lower waist-to-hip ratio than did GADab- men (Table 2).

Association between GADab levels and clinical characteristics. There was an inverse correlation between the FS C-peptide concentration and the GADab level (R = –0.25, P = 0.029). As shown in Fig. 2, the GADab+ patients differed only slightly from the low GADab+ group with respect to C-peptide concentration (median 0.62 vs. 0.55 nmol/l, P = 0.045), whereas the high GADab+ patients had significantly lower FS C-peptide levels (0.27 nmol/l, P = 0.0001). Only the high GADab+ group differed from the type 2 diabetic patients with respect to BMI (Fig. 2) and insulin response during the OGTT (data not shown).

FIG. 1. The prevalence of GADab in control subjects, subjects with IGT, and type 2 diabetic patients divided into subgroups according to age at onset. P < 0.0001 for the difference between type 2 diabetic patients and control or IGT subjects. P < 0.0001 for the difference between diabetic patients ≤45 years old compared with those ≥45 years old at onset of diabetes.
A different picture was seen with respect to the metabolic syndrome. Both the patients with high and low GADab+ levels differed from the GADab− patients with respect to lower blood pressure (systolic 141.3 [28.3] vs. 149.2 [30.5] mmHg, P = 0.009; diastolic 78.8 [17.2] vs. 81.0 [13.1] mmHg, P = 0.004), lower fasting C-peptide (0.08 ± 0.11 vs. 0.53 ± 0.39, P = 0.005), lower serum triglyceride concentrations (0.82 ± 0.11 vs. 1.67 ± 0.86, P = 0.009). However, the frequency of type 1 diabetes susceptibility genotypes, either DQB1 or insulin gene, did not differ from the rest of the GADab+ patients. Thus, the GADab+ patients who had been assigned permanent insulin treatment at onset of diabetes clinically resembled type 1 diabetes more than the other GADab+ patients did.

**Genetic comparison between GADab+ and GADab− individuals**

**IDDM1: HLA-DQB1 genotypes.** The proportion of subjects with the type 1 susceptibility allele *0302 was twice as high in GADab+ type 2 diabetic patients (41%) as in both GADab− patients (21%) and control subjects (24%) (P = 0.007). As shown in Table 3, both 0201/0302 and 0302/X genotypes were increased in frequency in GADab+ patients compared with GADab− patients and control subjects (0201/0302: 13 vs. 4 vs. 4%, P = 0.002; 0302/X: 22 vs. 12 vs. 12%, P = 0.010). No difference was seen in the frequency of genotypes comprising the *0602(3) allele considered protective from type 1 diabetes (Table 3).

### TABLE 2

<table>
<thead>
<tr>
<th>Clinical characteristics of the subjects</th>
<th>Control subjects</th>
<th>IGT subjects</th>
<th>Type 2 diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>GADab−</td>
<td>GADab+</td>
<td>GADab−</td>
<td>GADab+</td>
</tr>
<tr>
<td>n (M/W)</td>
<td>366 (168/198)</td>
<td>538 (231/207)</td>
<td>20 (5/15)</td>
</tr>
<tr>
<td>Age at onset of diabetes (years)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>50.4 (20.5)</td>
<td>59.7 (23.3)</td>
<td>5.7 (0.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 (4.5)</td>
<td>26.8 (5.0)</td>
<td>27.6 (5.6)</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>126.7 (21)</td>
<td>139.3 (27)</td>
<td>148.0 (26)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.0 (14)</td>
<td>81.0 (13)</td>
<td>81.0 (13.1)</td>
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<td>Waist-to-hip ratio</td>
<td>0.93 (0.07)</td>
<td>0.96 (0.08)</td>
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<tr>
<td>HDL cholesterol</td>
<td>1.20 (0.15)</td>
<td>1.20 (0.15)</td>
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<tr>
<td>Triglycerides</td>
<td>1.38 (0.43)</td>
<td>1.47 (0.70)</td>
<td>1.23 (0.45)</td>
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<tr>
<td>Cholesterol</td>
<td>2.50 (5.7)</td>
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<td>2.50 (5.7)</td>
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</tbody>
</table>

Data are medians (interquartile ranges). For insulin area, n = 544 for type 2 diabetes, and n = 54 for LADA. Data are corrected for BMI, duration, age, and FBG.
The frequency of genotypes comprising the *0302 allele was significantly lower in GADab* type 2 diabetes than in type 1 diabetes (41 vs. 71% P < 0.0001). This was mainly due to a lower frequency of the 0201/0302 genotype in the GADab* type 2 patients (13%) compared with type 1 diabetic patients, either young-onset (34%) or adult-onset (41%) (Table 3). The difference in the frequency of 0302/X between GADab* type 2 (22%) and young-onset (30%) or adult-onset (25%) type 1 diabetes was not statistically significant.

Genotypes comprising the protective allele 0602(3) were rare in type 1 diabetic patients, whereas GADab* type 2 patients did not differ from control subjects (Table 3). Thus, the proportion of subjects with 0602(3) was significantly lower in type 1 diabetes (17% in Botnia) than in GADab* type 2 diabetes (36%, P ≤ 0.009). No significant difference was observed between the young- and adult-onset type 1 diabetic patients (18 vs. 13%).

In both type 1 diabetes and GADab* type 2 diabetes, the frequency of genotypes comprising the 0201 allele was increased only in combination with the high-risk allele 0302. **Association between HLA DQB1 alleles and GADab levels.** Figure 2 shows the frequency of type 1 high-risk genotypes 0201/0302 and 0302/X stratified according to tertiles of GADab levels. Among type 2 diabetic patients, the frequency of the high-risk genotypes increased along with higher GADab levels: 15% in GADab* patients versus 29% in patients with low GADab levels versus 47% in patients with high GADab levels (P = 0.0011; df 2).

**IDDM2: The frequency of restriction fragment length polymorphism genotypes of the Hph1-polymorphism in the insulin gene.** The GADab* type 2 diabetic patients did not differ from the GADab* patients or control subjects with respect to the frequency of the +/+ genotype associated with type 1 diabetes (47 vs. 52 vs. 55%). The results were similar when subjects with the DQB1 high-risk genotypes 0201/0302 and 0302/X were excluded (data not shown). As expected, the frequency of the +/+ genotype was high in the type 1 diabetic patients with young (82%) or adult (69%) onset in the Botnia Study (P ≤ 0.0002 vs. all other groups).

**DISCUSSION**

The WHO group on the classification and diagnosis of diabetes acknowledged LADA as a separate entity by dividing type 1 diabetes into an autoimmune and idiopathic form. The former was further subdivided into a rapidly (classic type 1 diabetes) and slowly (LADA) progressive form. Unfortunately, there is no consensus regarding diagnostic criteria of LADA. This study was undertaken 1) to establish the prevalence of GADab* patients among Finnish type 2 diabetic patients, 2) to clinically and genetically characterize this subgroup, and 3) to use this information to provide a definition for LADA. The subjects participated in the Botnia Study, which is a population-based type 2 diabetes family collection study in western Finland (27). Several subjects were diagnosed with diabetes during the OGTT at the examination. Thus, the present study covers most diabetic patients in the area. According to the revised WHO and American Diabetes Association criteria, an FBG of 6.1 mmol/l was used to define diabetes (28). Five percent of all patients with diabetes (53/1,122) and 4% of the GADab* patients (4/104) would have been classified as nondiabetic (50 as IGT and 3 as normal glucose tolerant) using a FBG level of 6.7 mmol/l as diagnostic.

**FIG. 2. Comparison of patients with type 2 diabetes (n = 1,121) who have no GADab (≤5 RU; n = 1,017), low GADab levels (>5–38 RU; n = 70), or high GADab levels (>38 RU; n = 34), and patients with type 1 diabetes (n = 194). Data are shown as means ± SE; M, males; F, females. Significant differences between groups (P < 0.05) are indicated with an asterisk. FS C-peptide: P = 0.045 for GADab* vs. low GADab*; P = 0.0001 for low GADab* vs. high GADab* and for high GADab* vs. type 1 diabetes. BMI: P = 0.030 for low GADab* vs. high GADab*. Diastolic blood pressure: P = 0.030 for GADab* vs. low and high GADab*. Fasting serum triglyceride concentration: P = 0.0086 for GADab* vs. low and high GADab*; P < 0.0001 for high GADab* vs. type 1 diabetes. FS HDL cholesterol: P = 0.08 for GADab* vs. low and high GADab* (females); P < 0.0001 for high GADab* vs. type 1 diabetes (females).**
for diabetes. However, using the WHO 1986 criteria did not change either the prevalence of GADab positivity or the clinical characteristics of GADab+ patients (data not shown).

Overall, 9.4% of the patients diagnosed with type 2 diabetes were positive for GADab. The prevalence of GADab was higher among patients with age at onset of diabetes between 28 and 45 years (19%), whereas it remained rather stable after 45 years (mean 8.2%, range 6.5–9.7%). These figures agree with the data from the UKPDS, where 34% of patients diagnosed before 35 years, 14% of those between 35–44 years, and 7–9% thereafter were GADab+ (7). The other pancreatic autoantibodies, ICA and IA2ab, were less frequent, and their presence was associated with GADab positivity and GADab levels. That they were detected in only 0.5% of the GADab− patients, a figure comparable to the 2% ICA positivity in GADab− patients in the UKPDS, suggests that screening for them in patients with type 2 diabetes would have a marginal effect, at most, on increasing the sensitivity to diagnose LADA.

As expected, the GADab+ patients were more insulin deficient than were the GADab− patients, as evidenced by lower fasting C-peptide concentrations and lower insulin concentrations during the OGTT. The lowest values were seen in the patients belonging to the highest tertile of GADab, whereas the patients with lower GADab levels had only marginally lower fasting C-peptide concentrations compared with the GADab patients. Similar to our previous results in Finnish patients (5), but differing from the UKPDS (7), the mean BMI and the age at onset of diabetes of the GADab+ patients differed only slightly from those in GADab− diabetes. Again, the differences were mainly due to the high GADab+ group. However, it should be kept in mind that even the high GADab+ group had significantly higher C-peptide levels than did patients diagnosed with type 1 diabetes. Of note, the “low” GADab positivity was hardly due to a technical problem since 29% of GADab+ type 1 diabetic patients participating in the Combined Autoantibody Workshop (33) had GADab levels comparable to those referred to as “low” in this study.

The metabolic differences between GADab+ patients, GADab− type 2 diabetic patients, and type 1 diabetic patients were reinforced by the finding that the GADab+ patients also differed genetically from the two classic diabetes types. In the GADab+ type 2 patients, the frequencies of high-risk HLA-DQB1 genotypes, 0201/0302, and other genotypes with 0302, were significantly lower than in patients with type 1 diabetes but higher than in GADab− patients with type 2 diabetes. The frequency of the high-risk genotypes (0201/0302 and 0302/X) in the type 1 diabetic patients from the Botnia study (65%) were similar to those reported from the Finnish DiMe study (68%) (38). Another feature distinguishing both type 2 groups from type 1 diabetes was normal, i.e., not decreased, frequency of the DQB1*0602(3) allele, which confers protection against type 1 diabetes. Moreover, the GADab+ type 2 patients did not show the expected increased frequency of IDDM2 (insulin gene Hph1 polymorphism) susceptibility genotype typical for type 1 diabetes. Homozygosity for the short variable number of tandem repeat alleles in the promoter region of the insulin gene—in linkage disequilibrium with the +/+ Hph1-genotype—is associated with an increased risk for IDDM in Caucasian populations (37,39). In contrast

### Table 3

<table>
<thead>
<tr>
<th>DQB1 genotype</th>
<th>Control</th>
<th>GADab−</th>
<th>P1</th>
<th>GADab+</th>
<th>P2*</th>
<th>Type 1 diabetes</th>
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<tbody>
<tr>
<td>0201/0302</td>
<td>7 (4)</td>
<td>7 (4)</td>
<td>0.002 (0.0012)</td>
<td>12 (13)</td>
<td>0.0004 (0.002)</td>
<td>30 (37)</td>
</tr>
<tr>
<td>0201/0602(3)</td>
<td>23 (13)</td>
<td>22 (12)</td>
<td>—</td>
<td>10 (11)</td>
<td>—</td>
<td>1 (1)</td>
</tr>
<tr>
<td>0201/X</td>
<td>26 (15)</td>
<td>37 (19)</td>
<td>—</td>
<td>13 (14)</td>
<td>—</td>
<td>11 (13)</td>
</tr>
<tr>
<td>0302/0602(3)</td>
<td>13 (8)</td>
<td>11 (6)</td>
<td>—</td>
<td>6 (6)</td>
<td>—</td>
<td>5 (6)</td>
</tr>
<tr>
<td>0302/X</td>
<td>21 (12)</td>
<td>22 (12)</td>
<td>0.010 (0.060)</td>
<td>21 (22)</td>
<td>—</td>
<td>23 (28)</td>
</tr>
<tr>
<td>0602(3)/X</td>
<td>51 (30)</td>
<td>51 (27)</td>
<td>—</td>
<td>20 (21)</td>
<td>0.036 (0.216)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>X/X</td>
<td>31 (18)</td>
<td>41 (22)</td>
<td>—</td>
<td>13 (14)</td>
<td>—</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Total n</td>
<td>172</td>
<td>191</td>
<td>—</td>
<td>95</td>
<td>—</td>
<td>82</td>
</tr>
</tbody>
</table>

Data are n (%). P1 GADab+ type 2 diabetic vs. GADab− type 2 diabetic and control subjects; P2 GADab+ type 2 diabetic vs. type 1 diabetic subjects. P values in parentheses are corrected for multiple comparisons.
to the frequency of 71% in both children and adults with type 1 diabetes in the Botnia study, which compares with 76% reported in the DiMe study (40,41), only 53% of GADab+ type 2 patients had the +/+ Hph1-genotype.

Is the different genetic background of GADab+ type 2 diabetes simply an age-related phenomenon? This could explain the diversity of type 1 diabetes in the 30% of patients whose disease is diagnosed after the age of 35 years. Among young-onset type 1 diabetes, the prevalence of 0201/0302 and DR3/DR4 seems to be age-related (42,43). Also, compared with younger patients, those older than 20 years at onset of type 1 diabetes have been reported to be less often heterozygous for DQB1*0201/0302 (20%) (42) and DR3/DR4 (12.5–24%) (44,45). However, in our study, no difference in genotype frequencies was observed between the young-onset and adult-onset type 1 diabetic patients. Of note, the frequency of DQB1*0201/0302 was lower in GADab+ type 2 patients (12%) compared with both young-onset (34%) and adult-onset (41%) type 1 patients in the Botnia study, although it did not differ from the frequency of DR3/DR4 in adult-onset patients in another Finnish study (12.5%) (45). Also, the proportion of GADab+ type 2 patients having genotypes comprising DQB1*0302 or 0602(3) was clearly different from type 1 diabetes, whereas no difference was observed in the genotype frequencies between the two type 1 diabetic groups. This confirms previous data that age at onset of diabetes does not affect the frequency of either the protective DR2 and DQB1*0602(3) or the susceptibility-increasing DR4 and DQB1*0302 (44,45). However, in contrast to the studies mentioned above, a recent small study from Germany reported that only 17% of 24 adults over 40 years old at onset of type 1 diabetes had DQB1*0302, whereas 21% had DQB1*0602 (46).

It is unclear whether these frequencies could be affected by the low antibody positivity rate in the study patients. All in all, it seems that the prevalence of the DQB1*0201/0302 (DR3/DR4) genotype may reflect the age at onset of (autoimmune) diabetes, whereas the 0302 genotype appears to be associated with type 1 diabetes and only marginally affected by the age at onset. Of note, there are no age-related differences in the genotype frequencies in the normal population. Whether these age-dependent differences reflect a longer or repeated exposure to environmental influences remains to be shown. The ultimate answer to this question would be to identify and compare LADA patients with a sufficient number of adult-onset patients with rapidly progressing autoimmune diabetes requiring insulin treatment from the beginning. Unfortunately, these patients seem to be rare, even in Scandinavian countries with a high incidence of type 1 diabetes.

The third aim of the study was to provide a definition for LADA. We have shown that with respect to IIDD and IIDD2 type 1 diabetes susceptibility loci, GADab+ patients differ genetically from both type 1 and type 2 diabetes. Further, GADab levels above 5 RU distinguish a subgroup of type 2 diabetic patients characterized by fewer features of the metabolic syndrome. Within this group of GADab+ patients, those with high GADab levels (≥38 RU or the highest tertile) are also characterized by a significant impairment in their β-cell function. GADab+ type 2 diabetic patients differed from patients with classic type 1 diabetes by having higher C-peptide concentrations and more features of the metabolic syndrome. Only 3% of type 2 diabetic patients were diagnosed before the age of 35 years, whereas 70% of type 1 diabetic patients are diagnosed before that age. Given that only 5% of all LADA patients were diagnosed before the age of 35 years and the median age at diagnosis was 58.5 years, it may be reasonable to define LADA as GADab positivity (≥5 RU) in patients with an age at onset of diabetes of ≥35 years and who do not initially (at least 6 months) require insulin.

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Q2: In Fig. 2, please designate what asterisks refer to and add in legend in appropriate places.
Q3: Please spell out VNTR

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